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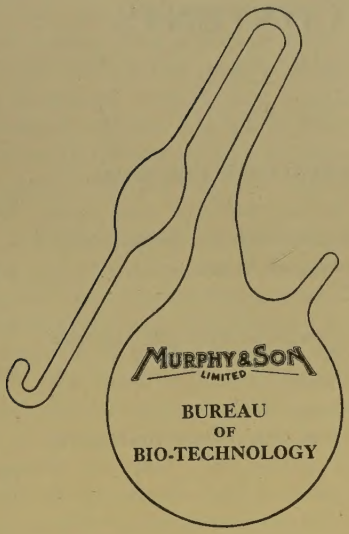

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The Effect of Pressure on Yeast Cells with some Remarks thereon

ATTENTION has been directed by E. R. Moritz[1] to the altered configuration of outline which occurs in yeasts that have been subjected to pressure such as is operative during filtration by filter-press methods now much in vogue. The same author has pointed out[2] that a simple modification of the technique usually employed in the examination of brewery yeasts is necessary in order to observe the phenomenon. There appears to be still a number of brewers who have not yet seen yeast showing the hexagonal shape and compact disposition assumed by the cells after subjection to such treatment, but it is a simple matter to make a mount of pressed yeast representing a sample from the ice chest in which it is customarily stored. To do this, one transfers to a microscope slide a small portion of the yeast broken off from a larger sample. With a second slide, held so that the bottom edge of its free end rests upon the first slide at an angle of about 20° and just touching the left side of the specimen, the material is now drawn out with slightly increasing pressure. This results in the production of a film which tails off towards the right to extinction. An $\frac{1}{8}''$ objective may be used for this examination preferably without covering the film, although, if desired, one may use a dry cover glass which should be lowered on to the film with great care in order to prevent any possibility of sliding, which would ruin the preparation. The shape and arrangement of the cells persist if the film thus prepared be dried, fixed and stained. Safranin is a good stain to use for this purpose, after which the preparation may be mounted in

[1] *Journ. Inst. Brewing*, XXVII, 1921, p. 572.
[2] *Loc. Cit.*, XXXI, 1925, p. 9.

Canada balsam, or examined uncovered by means of a 1/12″ oil immersion lens.

In this connection I may remark on what I believe to be a new observation. One of the things that has happened as a result of the application of pressure has been to alter the configuration of the cells to identically that which applies in the ordinary way during mass growth as the result of internal cellular pressure. In other words, the yeast cells revert to a normal multicellular arrangement. If a colony of yeast cells develops whilst suspended in a liquid medium the cells are round, oval or of other curved forms. It is under similar conditions that we are in the habit of observing these cells, but I have found that in the case of a yeast developing on the surface of a solid medium the cells composing the mass have a more or less hexagonal arrangement and in such close juxtaposition with neighbouring cells as to exhibit no such interspaces as would be necessary were the cells of the shapes with which we are so familiar. This is an arrangement common in nature, and it not only results in the saving of space but also in strengthening the fabric or tissue so composed by means of the method mentioned above. I have examined the cells from colonies of various species of yeast and torulæ grown from single cells on solid culture media, and they all possess this feature.* It will thus be seen that so far from there being any detrimental significance in the arrangement of the cells due to pressure, the hexagonal appearance is normal to healthy yeast.

Now with regard to the effect of pressure filtration from the fermentation point of view. There is considerable diversity of opinion as to the value of the product as a pitching yeast so far as its attenuative and reproductive properties are concerned ; there can be no question as to the value of the quite extraordinary effect of freeing the yeast from bacterial contamination which results from at least one system of pressure filtration and subsequent storage in solid form at low temperatures. Some brewers find that they are unable to successfully attenuate their beers with

* This fact was demonstrated at a meeting of the North Eastern Section of the Institute of Brewing, held at the Queen's Hotel, Leeds, Feb. 26th, 1925.

the pressed yeast and that there is a corresponding diminution in outcrop. The explanation usually offered is that pressure has resulted in a devitalization of the cells, and in some cases it has proved so drastic as to cause rupture. I have seen in pressed yeast, cells which appear to have been thus ruptured and disorganised, but never in such numbers as would lead to the belief that on this score alone reproduction would be appreciably impaired, whilst, at the same time, I have been aware that the yeast did not behave in the tun as microscopical appearances would warrant one to expect.

We are not without experimental evidence that yeasts and other micro-organisms can withstand enormous pressure without their reproductive power being destroyed. In a research by Hite and his collaborators on the effect of pressure on certain micro-organisms encountered in the preservation of fruits and vegetables, a number of organisms were submitted to pressures varying up to 120,000 lbs. per square inch. The following species were used in the investigation : *Bacillus prodigiosus* (Ehrenberg) Flügge, which occurs commonly in water, milk, bread and other starchy foods with the production of a blood-red pigment ; *B. fluorescens liquefaciens* Flügge, a common soil and water organism ; *B. subtilis* F. Cohn, the common spore-forming hay bacillus ; *B. lactis ærogenes* Hüppe and *Streptococcus lacticus* Kruse, two milk organisms ; *Saccharomyces cerevisiæ* Meyer ; *Saccharomyces albicans* Reess, a yeast parasitic in the throats of infants and young animals and the causative agent of the disease known as thrush ; and the pathogenic bacilli, *typhosus* Eberth., and *diptheriæ* Löffler. All these organisms showed good reproductive power after submission to pressures of not less than 20,000 lbs. per square inch, whilst some of them, *B. subtilis* for instance, developed after 30 minutes at 90,000 lbs. pressure. In the case of *S. cerevisiæ*, with which we are more directly concerned, cells were tested in distilled water, in 3 per cent. saccharose broth, 3 per cent. dextrose broth, 3 per cent. lactose broth and 3 per cent. maltose broth respectively. The organisms were afterwards cultivated upon 1 per cent. dextrose. In distilled water a pressure of 50,000 lbs. for 10 minutes in one case did not result in death, whilst after 25,000 lbs. for 140 minutes,

cells still exhibited vitality. Similar results were obtained in the sugar solutions although the pressure death-point was somewhat lowered. In maltose there was good growth after 20,000 lbs. for periods of time varying from 10 to 18 minutes.

Otto Rahn[3] states that *B. anthracis*, *B. pseudodiptheriæ*, *M. pyogenes* var. *aureus*, *Oidium lactis* and *Saccharomyces cerevisiæ* survive a pressure of 2,000 atmospheres for 96 hours, and points out that a specific difference between the moulds, yeasts and bacteria in this particular does not seem to exist. In works practice the pressure to which yeast is subjected approaches nothing like that mentioned above, and it seems reasonable to conclude that pressure *per se* is not responsible for loss of vigour in pressed yeast.

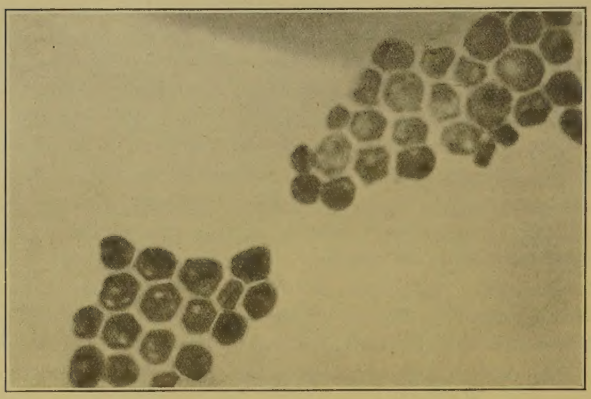


FIG. 31.

YEAST CELLS FROM A SAMPLE OF PRESSED YEAST.

The interspaces shown in the photograph are the result of drying, before staining; in the moist condition the cells have a compact, honey-comb appearance.

[3] *Marshall's "Micro-Biology,"* 3rd Edition.

The experiments to which reference has been made were carried out on cells suspended in liquid media. Rahn, in discussing the influence of mechanical agencies on micro-organisms in general, says that "if liquids, instead of being exposed to direct pressure are exposed to compressed air, the situation is entirely different. In this case the effect of the gas is the deciding agent. The fatal pressure under these conditions will vary as much as the fatal dose of an antiseptic; it depends on the chemical quality of the gas, upon the temperature and upon the organism." It is known that carbon dioxide at a pressure of 50 atmospheres retards the growth of bacteria in water and will sterilise it in 24 hours. Is it not possible that pressure plus the action of carbonic acid gas with which the liquid is charged and in which the cells are immersed during pressure in our brewery practice is at the root of the difficulty sometimes realised in the use of pressed yeast?

F. A. Mason.

Production of Sound Beer with the minimum of Preservative

IT is evident from our correspondence that the effect of the regulations in regard to the use of preservatives in food stuffs, particularly beer, is beginning to be realised by the brewers.

Very few brewers find it necessary to use preservatives during the winter months, and it can be imagined the condition of plant and premises which necessitate such aids at that time. How will such places go on in the summer months with the to them niggardly allowance of five grains per gallon of SO_2, which is represented by the use of less than one ounce per barrel of Potassium Metabisulphite, without counting the amount which will already exist in the beer at the time of racking, or added afterwards with the finings? To comply with the order it will rarely be safe to add more than $\frac{3}{4}$ oz. P.M.S. per barrel, and in many cases half an ounce will be the limit.

Some few concerns will not be in the least perturbed at the new order of things, as they have been able hitherto to carry on quite comfortably without the aid of preservatives of any description, even in the hottest weather; but the majority of brewers have usually found it advisable to play safe in the summer months by adding from one to two ounces of preservatives per barrel, and even then have not been able to escape altogether from that bane of the brewer's life, "Returns."

It is usual to judge the success, or otherwise, of the operative brewers' efforts by the percentage of "returns," and where this can be kept below one per cent. all is acknowledged to be well, but it is not often realised that where one per cent. of the output

is actually returns there had been probably five or ten per cent. of the beer sold in an unsatisfactory condition. Some brewers pride themselves on being able to count their returns in gallons per month, although their output may be thousands of barrels in that time, whereas, others with an output of as many hundreds can depend on more barrels being returned during the few summer months than can safely be disposed of during the remainder of the year by the ordinary and legitimate method of neutralising and blending. The Excise figures of the last few years tell a sorry tale of the enormous waste of money represented in the figures for duty repaid on beer destroyed through having become unsaleable. Part of this loss may be attributed to faulty materials, where, as it is in some breweries even to-day, the buying is done without sufficient analytical control, and without the advice of the operative brewer, who is thus often expected to do the impossible—*i.e.*, sending out a sound product from unsound materials—but the explanation for by far the larger proportion of returns is to be found in the condition of plant and premises.

The new regulations will render it necessary to improve plant conditions in order to avoid contamination, rather than to rely on preservatives to mitigate the effect of such contamination. Many a local beer depends for its popularity on a particular fruity bouquet derived from organisms in the timber or floors, and whilst this may be quite pleasant in small amounts its development must be kept under strict control, otherwise the beer very soon becomes rank.

Local flavours are usually derived from slight traces of infection, and rarely from differences in materials or processes as is sometimes thought. These local flavours, although perhaps appreciated by regular customers, are very quickly noticed and objected to by strangers, and so are not conducive to expansion of trade. In one brewery, somewhat noted for the peculiar pineapple flavour of its beers and notorious for bad beer in the summer, several thousand yards of flagged cellar flooring were removed and replaced by concrete, with the result that the pineapple flavour disappeared and the summer troubles were greatly reduced. At least 12 inches of pestilential filth had to be removed and

replaced by clean ballast before the concrete was laid. The fermentation or putrefaction of this filth was always noticeable at week-ends, after the Saturday clean-down, by each flag becoming outlined in froth. Further attention to the walls, stagings, vessels, casks and utensils ultimately resulted in the elimination of the local characteristics of the beer and at the same time resulted in the production of a good, sound beer, which wonderfully improved the firm's summer reputation, but unfortunately the benefits gained by that particular piece of enterprise have since been lost by poor skill in the choice of materials, which for some considerable time have resulted in the production of a beer which, although sound, showed a very poor response to action finings. This latter trouble need not be enlarged upon here, as it is not one which will be very largely influenced by the presence or otherwise of preservatives, although it must be stated that many of the cases of stubbornness arise from the breakdown of badly modified material, as a result of bacterial or other action.

The presence of substances inimical to bacterial life—*e.g.*, Salicylic acid—often prevents the premature breakdown of complex nitrogenous matter in a colloidal form, in which condition ordinary finings seem incapable of exerting their usual clarifying action. It is not our intention to go further into the question of colloidal troubles in this article, other than to say that there is an immense field for investigation on this subject, and we have recently added to our staff a colloid expert with a view to the elucidation of the more intricate problems associated with haze in beers and worts.

Colloid chemistry is a relatively new science, but by its means many physiological and chemical phenomena have been explained, and there is every hope that many of the hitherto unsolvable problems connected with hazy beer will disappear when the knowledge gained in other industries has been applied and augmented by investigations in the brewing industry.

Many industries have profited by the researches carried out by investigators in fermentation problems, and it now remains to be seen whether we can profit by applying knowledge gained in other spheres to our own difficulties. We do not mean to imply

that there has been no work done in this direction, but a careful search through the bibliography of the subject fails to reveal any really connected effort to solve problems which in some of the other industries have received the attention of colloid experts would appear fairly simple. There are two possible reasons for this state of affairs—firstly, that the problems have not been brought sufficiently to the notice of colloid investigators ; secondly, that the brewing school curriculum has not included sufficient training in this branch of science, with the result that it has failed to turn out its pupils with the necessary knowledge to enable them to tackle the problems with the advantage of a fuller knowledge of the actual brewing processes involved than can be possessed by an outside investigator.

We are quite aware that a few isolated quotations or references to work done could be brought in contradiction of our remarks, but we do not fear that these will be in sufficient volume to disprove our contention that the fermentation industry, particularly that portion of it which relates to beer, has been badly neglected, and has suffered accordingly so far as colloidal research is concerned.

There are, undoubtedly, many reasons for colloidal instability in beer, and the efficient control of all of them must for the time being be an impossible task, but research will surely give us the remedy for the few undesirable results manifested by this instability, either in the form of re-peptisation or, alternatively, complete peptisation, which will bring the organic matter involved into a condition to render it amenable to the action of finings, and, at the same time, have no harmful effect on the quality of the beer.

To return to the subject of antiseptics, the practical loss of these safeguards will render it necessary for many brewers to thoroughly investigate and improve the condition of their plant, etc. Brewers as a class are denied in certain quarters any claim to godliness, but that is no reason why they should fail in the matter of cleanliness, and by cleanliness is meant more than the copious use of hot water. Surface cleanliness may be obtained by this means, but the cleanliness necessary to produce and maintain the beer in a sound condition must go below the surface. Poracity

of material is the direct cause of much of the unsoundness in present-day beer, owing to the fact that this poracity tends to harbour wild yeasts and putrifying bacteria, which, although not noticeable immediately a vessel or cask is cleaned, come into action shortly after filling, often with disastrous results to the contents. Obviously, then, the only way to ensure perfect cleanliness is to use non-porous material, or to render whatever materials are used non-porous by a suitable lining which will fill up the pores and present a perfectly smooth surface, which is easily kept clean. There is a difference of opinion amongst brewers as to the advisability or value of linings, and one of the main objections is that certain desirable qualities are imparted to the beer by maturing in wood. This claim is undoubtedly correct, subject to the qualifications mentioned previously, but in many cases the quality so imparted are far from beneficial, and if not carefully controlled may lead to considerable trouble.

Much of the beer exported to-day is sent out in lined casks, the brewer preferring to depend on the qualities brewed into the beer, rather than be at the mercy of the uncontrolled organisms existing in the pores of the most efficiently cleansed, unlined casks. Another objection to lining is that it is apt to flake off : this can only happen as a result of faulty application, either by insufficient attention to the drying of the timber or by attempting to cover scale or fust.

Beer scale contains large numbers of yeasts and other organisms whose subsequent development is certain to lift whatever lining is used, and the same argument applies to fusty timber. Many brewers who have tried and been dissatisfied with the results of lining have failed to achieve satisfaction through lack of attention in the preliminary preparation of the casks or vessels. It is hardly fair to expect any lining to remedy defects already existing, the chief claim to the manufacture being that a good lining applied to a sweet dry cask will prevent the cask from ever becoming foul, in addition to which the cost of cleaning same will be greatly reduced.

The best time to apply a lining is when the timber is new, after the necessary seasoning has been carried out to empty the pores, but where, as is often the case, the cask or vessel is an old one, perhaps even a stinker, then every precaution should be taken to thoroughly clean, sterilise and dry before attempting to apply a lining. Again, some of the cheap linings are liable to lose their nature and either become brittle or else disintegrate into a powder, which is apt to impart a disagreeable flavour to the beer, and it is hardly necessary to emphasise the importance of dealing with a firm whose experience enables them to choose the materials which will give the best results.

The difference in the cost of lining a cask with the best—as against the cheapest—lining never amounts to more than a few pence, a negligible amount considering the consequences from a defective material. In cask lining, as in any other commodity, although the dearest may not always be the best, a firm of repute will maintain its quality, even in face of fierce price-cutting by newcomers who know nothing of analytical control of materials, with their consequent safeguards to the brewer using the resulting product.

GEO. JONES.

pH Values and the use of the Comparator in Brewing

TO judge by the amount and nature of the matter on the subject of Hydrogen-ion Concentration now being published it is easy to persuade oneself that the brewer knows all about hydrogen ions, their role in the mash tun, their bearing upon the nutrition and reproduction of yeast, and on the keeping qualities of a finished beer. We made this mistake in the *Bulletin* in 1921. Correspondence and personal discussion with brewers since then has effectively dispelled the illusion, and we find that brewers are only now beginning to feel that they ought to know these things, and there is a very genuine desire on the part of a great many busy men to probe into the meaning of H.I.C. and the significance of pH values. A well-known member of the profession writes :—

> "I am a little inclined to take some interest in this subject if I could convince myself that it is really practical and useful. Have you a small, concise, practical book on the subject giving the useful purpose in view, the results obtained and the manipulation and process used in obtaining useful practical results. Most of what I have seen in periodicals on the subject has not been lucid or edifying—in fact it has been rather repellent."

One reason why our correspondent has found the treatment of the subject rather repellent is that he has begun at the wrong end. It is no more possible to interpret the illuminating results now being published in our own country by Hagues[1], Hopkins,[2] Parsons[3] and others without a grounding in the subject than it is

to decipher fascinating inscriptions on Egyptian tablets without a knowledge of hieroglyphics.

Is the consideration of pH Values worth while?

To refer first of all to the usefulness of the subject of hydrogen-ion concentration. Pasteur says, with reference to his work on the method of sterilisation now called Pasteurization, "It is easy to show that these differences of temperature which are required to secure organic liquids from ultimate change depend exclusively upon the state of the liquids, their nature, and above all upon the conditions which affect their neutrality, whether towards acids or bases." As has been pointed out by W. Mansfield Clark,[7] Pasteur had recognised phenomena in the behaviour of solutions for which he could find no explanation, and for which no satisfactory explanation was forthcoming until some fifty years later, after S. P. L. Sørensen,[6] working in the famous Carlsberg Brewery Laboratory, had given to the world his researches on hydrogen-ion concentration in relation to bio-chemical problems. How intimately associated with the present-day welfare of the brewer is this question of hydrogen-ion concentration and its measurement, is revealed by the work of N. M. Parsons[8] on pH values as criteria of stability. In this connection the possibilities of its usefulness to brewers may be gauged by the rather innocent-looking question set in the Institute of Brewing Examination, 1924, Part II., "What method has recently been proposed as an alternative to the forcing tray process for anticipating the keeping qualities of beer?"

In the study of bacteria, yeasts and moulds the relationship of hydrogen-ion concentration to growth, development, inhibition and even differentiation of species has been shown to be so intimate and, indeed, inseparable, that it has become a matter of paramount importance to the biologist. W. M. Clark[5] has indicated that it is one of the controlling factors in all living processes in his remark, "The rate of enzyme action, the stability of colloidal structures upon which life depends, the solubility of many physiologically important compounds, as well as the structure and

composition of media and the colour of the indicators used in the adjustment of their reactions, all are dependent in greater or less degree upon hydrogen-ion concentration." In brewing, in which almost everything depends upon the growth of microscopic living cells, the significance of hydrogen-ion concentration can no longer be ignored.

Before leaving the question of usefulness it may be pointed out that it is the hydrogen-ion concentration of the brewing water and the malt which controls the amount and quality of extract, the break, the attenuation and the fining property of beers. The brilliancy, condition and foaming property are each expressions of hydrogen-ion concentration, which can be measured and recorded as pH values.

Why are Old Methods Inefficient?

A beer that has become sour is said to have developed acidity. Living organisms, during the processes involved in their growth and physiological activities, have produced acetic acid which remains dissolved in the beer, to which it imparts its characteristic taste. For some purposes, the brewer, or his chemist, has occasionally to estimate by simple chemical means the amount or percentage of acidity. To do this he takes an aliquot portion of the beer and titrates with dilute alkali solution containing a known quantity of the alkali. To determine the point at which the acid is neutralised an indicator is used, and those most commonly found on the laboratory shelves are litmus, phenol-phthalein and methyl orange. If litmus is used, either in solution or as litmus paper, the addition of alkali is continued until a colour intermediate between red and blue is obtained, indicating that the beer is neither acid nor alkaline towards litmus ; it is said to indicate neutrality. If the so-called neutral solution of beer and alkali be checked against phenol-phthalein it will be found to be still acid, whilst if checked against methyl orange it will prove to be alkaline. Had a check against only one of the two latter indicators been made it could easily be believed that either too little or too much alkali had been added, but here is a state of affairs in which there appears to be good

evidence that the titration has been overdone as well as underdone ; it is also an indication that it is unsatisfactory to define the terms acid and alkali in relation to the colour reaction of any of the indicators mentioned. We have discovered that each has a different range of sensitivity to reaction and that reaction is evidently not the same thing as total acidity. As is now known, the range of reaction between the neutral point of methyl orange and the neutral point of phenol-phthalein, small as it seems, covers a range of hydrogen-ion concentration of such magnitude as to control the development of many micro-organisms ; for some it is a range in which growth is inhibited, whilst for others it provides optimum conditions for physiological activity. Hence titration methods do not give full information in regard to acidity and alkalinity ; they fail to give expression to that property of acids which is most important.

What is meant by Hydrogen-ion Concentration?

A basis for the terms " Acidity," " Alkalinity " and " Reaction " is furnished by the ionic theory, or theory of electrolytic dissociation. The assumptions of this theory, made by Arrhenius,[8] are—

(1) When an electrolyte is dissolved in water, its molecules are immediately more or less completely dissociated into smaller fragments, or ions, of unlike composition : $HCl \rightarrow H + Cl$.

(2) These ions are charged with electricity ; the ions of the one product are charged with positive, the ions of the other with negative electricity, the unit positive charge being equal in quantity, but opposite in kind to the negative charge : $HCl \rightarrow H^+ + Cl^-$.

(3) The dissociation is a reversible action, $HCl \rightleftarrows H^+ + Cl^-$. This equation can be typified as $H\,Ac \rightleftarrows H^+ + Ac^-$, where H is the hydrogen ion and Ac the acidic ion. For example, acetic acid dissolved in water may be represented by $CH_3COOH \rightleftarrows H^+ + CH_3COO^-$ in which the negative ion is

represented by a group of elements. When an electric current is passed through a solution containing ions the positive ion appears at the negative pole or kathode and is termed a kation : the negatively charged ion travels to the positive pole or anode and is known as an anion. Electrolytes are completely ionised at infinite dilution.

Acids of different kinds dissociate to different extent ; hydrochloric acid and some other mineral acids dissociate very strongly, or, in other words, more completely than organic acids. Acetic and lactic acids dissociate to quite a small extent, and on this account they are termed weak acids ; carbonic acid and boric acid are also weak acids. It should be observed that " strong " and " weak " are not synonymous with " concentrated " and " dilute," the latter terms having reference only to the amount of acid and not their property of ionisation.

Acidity, Alkalinity and Reaction.

It is to the positive hydrogen-ion in solution that the property known as acidity is due. A solution of acetic acid contains dissociated H^+, and CH_3COO^- ions, together with undissociated CH_3COOH, but it is the concentration of the H^+ ions which is a measure of the *effective acidity* or *reaction*.

Alkalinity is explained similarly. A base when dissolved in water dissociates into basic hydroxyl-ions, the base carrying a positive charge and the hydroxyl-ion a negative charge of electricity : $NaOH \rightleftarrows Na^+ + OH^-$. Here again it is to one of these, the hydroxyl-ion, that *effective alkalinity* is due. There are strong bases like sodium and potassium hydroxides, and weak bases, of which ammonia is an example.

It is stated in assumption (3) that electrolytes are completely ionised at infinite dilution. Those weak acids which dissociate to only a slight extent continue to undergo dissociation as dilution proceeds, and at any given dilution the concentration of hydrogen-ions produced is in accordance with the law of mass action. The ratio of the product of the concentration of the hydrogen-ions H^+, and the acidic ions Ac^-, to the concentration of the undissociated

HAc is known as the dissociation constant of that particular acid and is represented by the symbol K; that is $K=\frac{[H^+]\times[Ac^-]}{[HAc]}$. The same equation may be applied in the case of a base, $K=\frac{[B^+]\times[OH^-]}{[BOH]}$. Thus there is always a definite relationship between the ionised and the non-ionised molecules in solution.

As examples of the degree of dissociation exhibited by different acids and alkalis, in the case of a decinormal solution of the strong acid HCl, 91 per cent. of the hydrogen is dissociated; of the weak acid CH_3COOH only 1·3 per cent. A decinormal solution of the strong alkali NaOH dissociates to the extent of 84 per cent., whilst a corresponding solution of NH_4OH only to 1·4 per cent. As previously mentioned, it is the concentration of the free H^+ or the OH^- ions which imparts the characteristic properties of acidity or alkalinity; in other words, the degree to which a solution possesses either property is dependent upon the concentration of the free or available hydrogen ions or hydroxyl ions respectively.

Neutrality.

Now our universal solvent water is itself an electrolyte. It dissociates according to the foregoing equation, $HOH \rightleftarrows H^+ + OH^-$, but it does so to an exceedingly small extent. Although there is an almost infinitely large mass of undissociated HOH and an almost infinitesimal proportion of ionised HOH, the H^+ and OH^- ions are not without measurable effect, and it is to these that water owes its behaviour as a conductor of electrical current. Sørensen has determined electrometrically that one litre of pure water at 22°C contains one ten-millionth gram of hydrogen-ions: that is, the concentration of H^+ ions is $\frac{1}{10{,}000{,}000}$ gram per litre $=\frac{1}{10^{-7}}=10^{-7}$. As the molecules of HOH have been equally dissociated into H^+ and OH^- ions, the negative ions OH^- must also equal $\frac{1}{10{,}000{,}000}$ gram per litre, $=\frac{1}{10^{-7}}=10^{-7}$. Here we have a solution in which

the H^+ ions giving an acid reaction are perfectly balanced by OH^- ions (which would themselves impart an alkaline reaction), with the result that we have a state of *absolute neutrality*.

In all measurements of hydrogen-ion concentration pure water is taken as the standard.

The Expression, pH.

At any given temperature the product of the hydrogen-ion concentration and the hydroxyl-ion concentration is a constant. As the hydrogen-ion concentration at 22°C, as determined by Sørensen, is 10^{-7}, and as the hydroxyl-ion concentration is equal, the dissociation constant is $10^{-7} \times 10^{-7} = 10^{-14}$. At 18°C. it is $10^{-7.07} \times 10^{-7.07} = 10^{-14.14}$; it will tend towards simplification in what follows if the value 10^{-14} is retained.

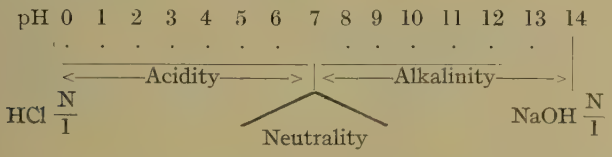
It has been stated that pure water always contains hydrogen and hydroxyl ions in balanced proportion. With so small a H^+ concentration as $\frac{1}{10,000,000}$ gram per litre, which is a readily measurable quantity, it may be easily conceived that it is extraordinarily sensitive to the presence of other dissociable substances, as it is. The absorption of traces of carbonic acid gas from the atmosphere immediately destroys the delicate balance of positive and negative ions owing to the production of more free H^+ ions, as will be seen from the following equation :—

$$CO_2 + H_2O \rightleftarrows H_2CO_3 \rightleftarrows H^+ + HCO_3^-$$

Such a solution would have a hydrogen-ion concentration greater than 10^{-7}, say 10^{-6}, and the solution would be no longer neutral. There would still be hydroxyl ions present, and their concentration may be calculated from the dissociation constant 10^{-14}; $\frac{10^{-14}}{10^{-6}} = 10^{-8}$.

It will be appreciated that whether use is made of the fractions $\frac{1}{10,000,000}$, $\frac{1}{100,000,000}$ *et seq.*, or their logarithmic equivalents 10^{-7}, 10^{-8} *et seq.*, it involves the manipulation of unwieldly figures,

which are altogether unmanageable in the production of graphs. Sørensen overcame this difficulty by adopting the symbol $\overset{+}{p}H$ to denote the "hydrogen-ion exponent" or "hydrogen-ion concentration." $\overset{+}{p}H$, or, as it is now more frequently written pH, is the logarithm to the base 10 of H^+ but with the negative sign omitted. Thus 10^{-7} becomes pH7; 10^{-3}=pH3; 10^{-13}=pH13, the numerical value of pH decreasing with increasing hydrogen-ion concentration. A scale illustrating the series of pH values from 0 to 14, *e.g.*, within the limits of the dissociation constant 10^{-14}, may be constructed as follows:—



The following table illustrates in another way the relation of pH values to the strength of hydrochloric acid and sodium hydroxide solutions[10]:—

TABLE I.

Strength of solution.	Grams of hydrogen per litre.	Expressed logarithmically.	PH value.
Normal HCl	1.0	10^{-0}	0·0
1/10 N HCl	0.1	10^{-1}	1·0
1/100 N HCl	0.01	10^{-2}	2·0
1/1000 N HCl	0.001	10^{-3}	3·0
1/10000 N HCl	0.0001	10^{-4}	4·0
1/100000 N HCl	0.00001	10^{-5}	5·0
1/1000000 N HCl	0.000001	10^{-6}	6·0
Pure water	0.0000001	10^{-7}	7·0
1/1000000 N NaOH	0.00000001	10^{-8}	8·0
1/100000 N NaOH	0.000000001	10^{-9}	9·0
1/10000 N NaOH	0.0000000001	10^{-10}	10·0
1/1000 N NaOH	0.00000000001	10^{-11}	11·0
1/100 N NaOH	0.000000000001	10^{-12}	12·0
1/10 N NaOH	0.0000000000001	10^{-13}	13·0
Normal NaOH	0.00000000000001	10^{-14}	14·0

Buffer Action.

So far we have considered only the behaviour of acids dissolved in water in the absence of any substance which might interfere with the full activity of their dissociated ions. A great many substances have the property of attracting to themselves hydrogen-ions which are then, of course, no longer free; such substances are called "buffers." They are, in point of fact, reaction regulators which prevent rapid changes in the hydrogen-ion concentration upon the addition of acids and alkalies. Many salts, particularly acetates, citrates, borates, phthalates and phosphates exhibit this property to a marked degree, whilst amino acids, peptones, beef extract, malt extract, gelatine and other complex organic substances act in the same way. In contrast with the rapid alteration in pH value which would occur in the case of absorbtion of CO_2 from the atmosphere by pure water, a buffered solution may remain exposed to air for a considerable time without undergoing any change. Any noticeable alteration in the latter circumstances would be almost certainly traceable to the activity of micro-organisms. Similarly, the addition of acetic acid in small quantities to pure water would have immediate effect on hydrogen-ion concentration and the pH value would be considerably lowered. If the same quantity of acid were added to a solution of sodium acetate, the difference in *reaction*, or available acidity, before and after addition would be only slight, although the *total acidity* would be the same in each case. Hopkins[2] has given an interesting example of the buffer effect of a 1 per cent. peptone solution on the reaction of lactic acid. 100 c.c. of pure water is taken, the hydrogen-ion concentration of which is $10^{-7\cdot07}$ (say pH 7). The effect of adding 1 c.c. of lactic acid will be to increase the hydrogen-ion concentration to $10^{-3\cdot42}$ (say pH 3·4), which represents an increase to about 4,400 times as much. If, however, the 1 c.c. of N/10 lactic acid be added to 100 c.c. of a 1 per cent. peptone solution of pH 7, the hydrogen-ion concentration is merely altered to pH 6·7, an increase to 2·34 times as much. Further, if the same quantity of acid be added to a 5 per cent. solution of peptone the increase is still smaller, about 1·2 times.

The estimation of total acidity by titration takes no account of *effective acidity* or *reaction*, whether controlled by buffers or not.

The Determination of pH Values.

There are two practical methods available for the determination of hydrogen-ion concentration. The more exact, where applicable, is the electrometric method. This is based upon the method of measuring differences in electromotive force set up by the presence of metallic ions (hydrogen functioning as a metal) in aqueous solution according to the concentration of ions. It involves the use of delicately-constructed and expensive apparatus as well as skilful manipulation. This method and its applications in brewing are lucidly described and discussed by N. M. Parsons in his paper,[3] which should be consulted.

Indicators.

The other method, known as the colourimetric method, depends upon colour changes produced in certain substances by varying acidity or alkalinity. These substances are called *indicators*, and they are sensitive to changes of reaction within quite restricted and very definite limits. To refer once more to those mentioned at an earlier stage. Litmus, although it has in the past been found so useful, is not a definite compound and its colour reactions vary: its neutral point lies between pH 6·6 and pH 7. Phenol-phthalein gives accurate results over its range, which is between pH 8·0 and pH 9·6, and which, as will now be understood, is considerably on the alkaline side of neutrality. Methyl orange has a range from pH 3·1 to pH 4·4, this being very considerably on the acid side. Sørensen made a very careful study of the relation of indicators to the pH scale, and Mansfield Clark, Lubs, Cohen, Medalia, Gillespie and others have further experimented in the same direction. New indicators are being constantly added to the list of those found useful in practice, and some of these and their ranges of sensitivity to reaction are shown in the following table:—

TABLE II.

COLOUR CHANGES OF THE INDICATORS OF CLARK AND LUB AND OF COHEN.[12]

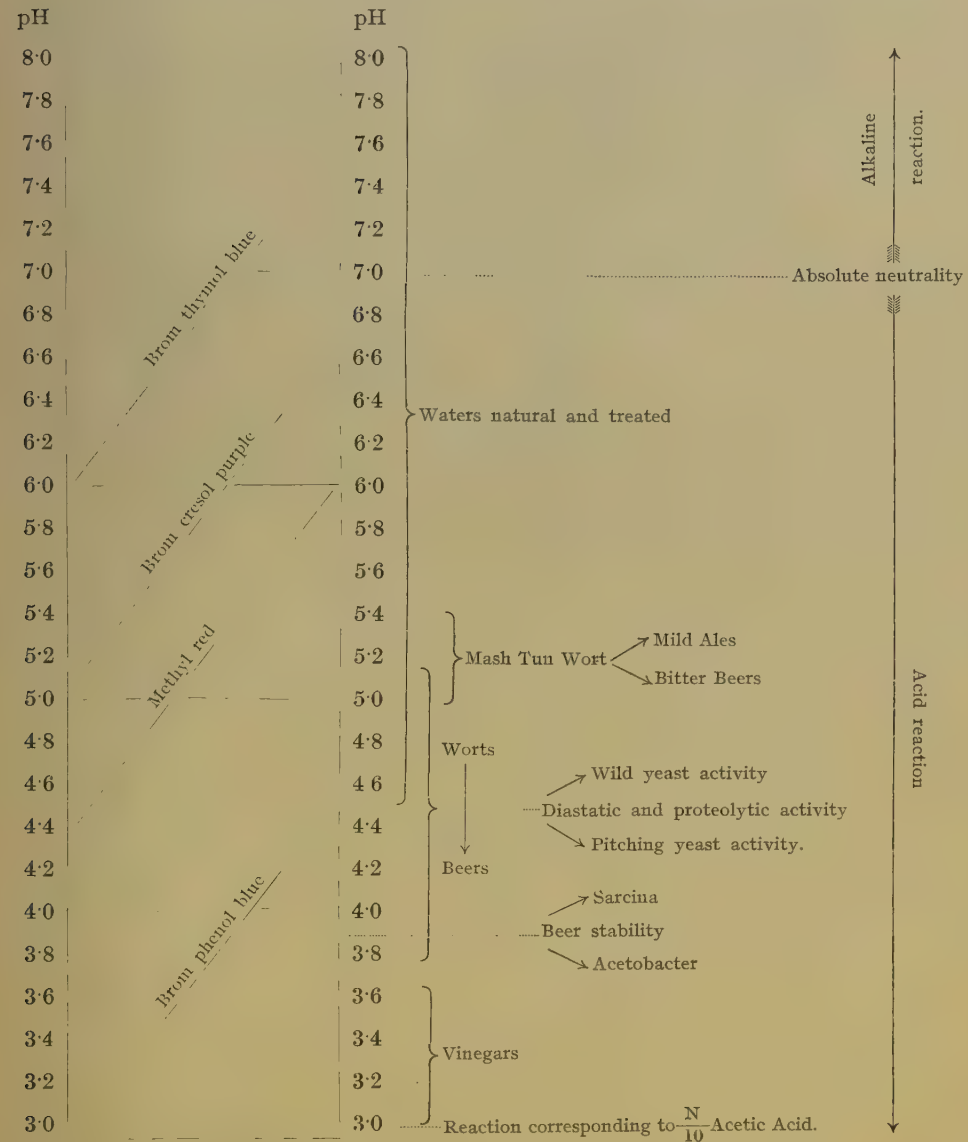
Indicator.	Full Acid Colour	Full Alkaline Colour	Sensitive Range
Thymol blue (acid range) ...	Red	Yellow	1·2–2·8
Brom phenol blue	Yellow	Blue	3·0–4·6
Brom chlor phenol blue	Yellow	Blue	3·2–4·8
Brom cresol green	Yellow	Blue	3·8–5·4
Methyl red	Red	Yellow	4·4–6·0
Chlor phenol red	Yellow	Red	5·0–6·6
Brom cresol purple	Yellow	Purple	5·2–6·8
Brom phenol red	Yellow	Red	5·4–7·0
Brom thymol blue	Yellow	Blue	6·0–7·6
Phenol red	Yellow	Red	6·8–8·4
Cresol red	Yellow	Red	7·2–8·8
Thymol blue (alkaline range) ...	Yellow	Blue	8·0–9·6
Phenol phthalein	Colourless	Red	8·0–9·6
Cresol phthalein	Colourless	Red	8·2–9·8

The indicators which have proved most useful in the brewing laboratory are Brom thymol blue, Brom cresol purple, Methyl red and Brom phenol blue.

Brom thymol blue is a particularly valuable indicator, as its range of sensitivity from pH 6 to pH 7·6 includes, of course, pH 7, taken as neutrality. At this point it gives a very definite grass-green colouration. It finds special use in adjusting micro-biological media, and, by using the " Biotec " comparator which is provided with a separate block for the purpose, a medium or solution of any kind may be adjusted *in situ* alongside a solution of the indicator and its colour compensation tube. Used for testing samples of water or other liquids, this indicator gives instantaneous information with regard to the acid, the absolute neutral, or the alkaline character of the sample ; it does not, of course, give a definite pH value unless the range of deviation from absolute neutrality lies within the range pH 6 — pH 7·6.

Brom cresol purple has proved more particularly useful in the examination of natural and treated waters. Methyl red and Brom phenol blue serve for worts and beers, although they are in frequent

INDICATORS APPLICABLE TO BROWING OPERATIONS



use for estimating the pH value of acid waters. The pH value of worts falls within the Methyl red range and that of beers within the range of Brom phenol blue. The use of the two last-mentioned indicators is not wholly satisfactory. It will be noted from Table II that there is an overlap of the less acid end of the range of Brom phenol blue with the more acid end of Methyl red, and with some samples of beer having pH values between 4 and 4·4, the pH indicated by Methyl red is not corroborated by the reaction indicated by Brom phenol blue. Methyl red is known to be unstable because of its tendency to undergo reduction. Brom phenol blue is liable to give trouble on account of its property of dichromatism.

The two indicators, Brom cresol green and Brom-chlor phenol red, included in our Table, with ranges covering the requirements of beer and wort, have only recently become available as the result of the investigations of Barnett Cohen.[12] Experiments are in progress with a view to determining their suitability for brewing work. The colour change of Brom cresol green is from yellow to blue-green in the direction of neutrality, whilst Brom-chlor phenol red shows a change in the same direction from yellow to blue.

The Comparator or Colourimetric Method.

This method has been fully described in *Bulletin* No. 3, Vol. 1, 1921, pp. 55-66, where full instructions for the preparation of standards are given. The "Biotec" Comparator has been illustrated and described in *Bulletin* No. 9, Vol. II, 1923, pp. 1-6, and a very useful and practical paper on the subject has been published by H. Lloyd Hind[11].

It is frequently a convenience to make use of a mixed indicator with which a solution may be roughly tested for its pH range before more accurately determining the point by the indicator best suited for the purpose. Such a solution is known as a Universal Indicator, and several of these are available. A useful one may be prepared as follows:—

Methyl Orange	0·04	gram
Methyl Red	0·02	,,
Naphthol-phthalein	0·12	,,
Phenol-phthalein	0·08	,,

Dissolve in 100 c.c.'s of 70% alcohol.

This gives the whole range of spectrum colours in the correct order from red (pH 3) to violet (pH 11), as follows (Predeaux and Ward) :—

Approximate Value of pH	Colour Reaction (5 drops indicator per 10 c.c. sample)
3	Crimson
4	Red
5	Orange red
5·5	Orange
6·0	Orange yellow
6·5	Yellow
7·0	Greenish yellow
7·5	Yellowish green
8·0	Green
8·5	Bluish green
9·0	Greenish blue
9·5	Blue
10·0	Violet
11·0	Reddish violet

It should be borne in mind that with this or any other indicator used in any coloured solution, the colour of the latter has to be taken into account. It is most conveniently examined by the Comparator alongside a tube of the sample to which no indicator has been added.

Practical Applications.

Water.—A perfect check can be kept upon the reaction of the brewing water by means of the comparator method. Well and river supplies are liable to considerable variation, especially those from limestone areas, and, according to N. M. Parsons, water delivered by the Metropolitan Water Board varies from day to day. Waters vary with regard to pH value within rather wide limits from pH 8 on the alkaline side to pH 4·5 on the acid side of neutrality. Peaty waters, such as are supplied by Northern Corporations, are always acid in reaction. Where the brewing water is treated, particularly in the case of acid treatment, the method constitutes a valuable check upon the materials used and on the rate of addition. In the routine examination of brewing waters we have found, for instance, in a case in which two liquor tanks of 120 barrels and 70 barrels

respectively were made up with proportionate quantities of treatment, with no greater variation than $\pm$ 0·1 pH, although the pH was designedly shifted pH 1·5 from that of the water from the rising main.

Brom Thymol Blue and Brom Cresol Purple are the most useful indicators for this purpose.

Mashing.—Hague and Hopkins have published results indicating the relation of hydrogen-ion concentration to mash tun processes. The optimum reaction for conversion appears to be pH 5·4. In our own practice we have found good results from mash tun worts of hydrogen-ion concentration between pH 5·0 and pH 5·3. Any rise or fall from these figures would be looked upon with suspicion.

Copper and Hop-Back for Wort.—Usually we have found the hydrogen-ion concentration decrease to the extent of 0·1 pH in the copper, although on the other hand an increase in reaction from pH 5·0 to pH 4·9 has resulted in the production of exceptionally stable beers. In the hop-back the wort, if it has decreased in pH in the copper, reverts to a reaction at least as acid as the mash tun wort, and at this stage the average lies between pH 4·8 and pH 4·9.

Fermentations.—In the F.V. the wort exhibits a rather rapid increase in hydrogen-ion concentration, quickly reaching pH 4·5, and continuing to increase up to racking. In our experience with samples of racking beer from a large number of sources, the racking pH lies between 3·9 and 4·1. We regard Parson's "Safety point" of pH 4·15 as being too low a hydrogen-ion concentration for average beers, and would say that pH 4·0 would be nearer the limit to be regarded as a "Safety point."

There is little doubt that each brewery will be found to have its own optima of pH values at the various stages of brewing, although there is unlikely to be great deviation from the figures mentioned above. The "safety point" not only of the finished beers, but at each stage of the process, is one that must be settled for each individual brewery.

F. A. Mason.

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APPENDIX.

As will be understood from our remarks on "buffers," it is desirable to have for reference a set of buffered solutions of known pH value. It was pointed out, in *Bulletin* No. 3, that by using Medalia's method of paired tubes, based on the "drop-ratio" of the acid and alkaline forms of the various indicators, the use of buffered solutions may be obviated. The buffer solutions require great accuracy in making up and much care in keeping, but as we frequently receive requests from users of our notes on Medalia's method for information as to their preparation, it seems desirable to include a list of the substances and quantities used. A number of mixtures have been devised by various workers. W. Maxwell Clark, "The Determinations of Hydrogen Ions" (Williams and Wilkins, Baltimore), 1922, gives a very complete list, p. 106-107, from which the following tables are copied. For further information on all the scientific aspects of the subject, his work should be consulted.

Composition of Mixtures giving pH Values at 20°C at intervals of 0·2.

From Clark (1922), *p.* 106-107.

KCl-HCl mixtures.

pH	M/5 KCl	M/5 HCl	Dilute to
1.2	50 cc.	64.5 cc.	200 cc.
1.4	50 cc.	41.5 cc.	200 cc.
1.6	50 cc.	26.3 cc.	200 cc.
1.8	50 cc.	16.6 cc.	200 cc.
2.0	50 cc.	10.6 cc.	200 cc.
2.2	50 cc.	6.7 cc.	200 cc.

Phthalate—HCl mixtures.

pH	M/5 KH Phthalate	M/5 HCl	Dilute to
2.2	50 cc.	46.70 cc.	200 cc.
2.4	50 cc.	39.60 cc.	200 cc.
2.6	50 cc.	32.95 cc.	200 cc.
2.8	50 cc.	26.42 cc.	200 cc.
3.0	50 cc.	20.32 cc.	200 cc.
3.2	50 cc.	14.70 cc.	200 cc.
3.4	50 cc.	9.90 cc.	200 cc.
3.6	50 cc.	5.97 cc.	200 cc.
3.8	50 cc.	2.63 cc.	200 cc.

Phthalate—NaOH mixtures.

pH	M/5 KH Phthalate	M/5 NaOH	Dilute to
4.0	50 cc.	0.40 cc.	200 cc.
4.2	50 cc.	3.70 cc.	200 cc.
4.4	50 cc.	7.50 cc.	200 cc.
4.6	50 cc.	12.15 cc.	200 cc.
4.8	50 cc.	17.70 cc.	200 cc.
5.0	50 cc.	23.85 cc.	200 cc.
5.2	50 cc.	29.95 cc.	200 cc.
5.4	50 cc.	35.45 cc.	200 cc.
5.6	50 cc.	39.85 cc.	200 cc.
5.8	50 cc.	43.00 cc.	200 cc.
6.0	50 cc.	45.45 cc.	200 cc.
6.2	50 cc.	47.00 cc.	200 cc.

KH_2PO_4—NaOH mixtures.

pH	M/5 KH_2PO_4	M/5 NaOH	Dilute to
5.8	50 cc.	3.72 cc.	200 cc.
6.0	50 cc.	5.70 cc.	200 cc.
6.2	50 cc.	8.60 cc.	200 cc.
6.4	50 cc.	12.60 cc.	200 cc.
6.6	50 cc.	17.80 cc.	200 cc.
6.8	50 cc.	23.65 cc.	200 cc.
7.0	50 cc.	29.63 cc.	200 cc.
7.2	50 cc.	35.00 cc.	200 cc.
7.4	50 cc.	39.50 cc.	200 cc.
7.6	50 cc.	42.80 cc.	200 cc.
7.8	50 cc.	45.20 cc.	200 cc.
8.0	50 cc.	46.80 cc.	200 cc.

Boric acid, KCl—NaOH mixtures.

pH	M/5 H_3BO_3 M/5 KCl	M/5 NaOH	Dilute to
7.8	50 cc.	2.61 cc.	200 cc.
8.0	50 cc.	3.97 cc.	200 cc.
8.2	50 cc.	5.90 cc.	200 cc.
8.4	50 cc.	8.50 cc.	200 cc.
8.6	50 cc.	12.00 cc.	200 cc.
8.8	50 cc.	16.30 cc.	200 cc.
9.0	50 cc.	21.30 cc.	200 cc.
9.2	50 cc.	26.70 cc.	200 cc.
9.4	50 cc.	32.00 cc.	200 cc.
9.6	50 cc.	36.85 cc.	200 cc.
9.8	50 cc.	40.80 cc.	200 cc.
10.0	50 cc.	43.90 cc.	200 cc.

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